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REMARKS

An Examiner Interview was conducted 19 Sep 2006. During the interview, the restriction requirement mailed 29 Jun 2006 was discussed and Applicants confirmed that they wished to pursue Group E, SEQ ID NO:25 under linking claim practice.

In their response to restriction, Applicants had requested that the Restriction be withdrawn, in-part, so that SEQ ID NO:10 is included with Groups A and B. This request was discussed as well and it is Applicants' representative's understanding that the Examiner agrees to this reformulation (which will not affect the present election under Group E, but rather subsequent divisional applications).

Applicants wish to thank the Examiner for the helpful interview.

During the interview, Applicants also agreed to file materials to clarify the relationship between the peptides discussed in the examples and the polypeptide fragment set forth in SEQ ID NOS:16-33. Accordingly, Applicants submit two Exhibits A & B. These exhibits are discussed in the following paragraphs.

Exhibit A contains pages 71-75 of US Serial No. 10/226872 (the '872 application), the parent of the present application. (The parent was incorporated by reference in the present application; see paragraph [0001] of the present specification.) Pages 71-75 of the parent '872 application comprise Example 10 and describe a series of in vitro priming experiments have been carried out with the PBMC's of three healthy donors to demonstrate that the CASB7439 antigen can generate a specific CD4+T-cell activity in human cells. The work was carried out using forty-five overlapping 15-mer peptides corresponding to the sequence of CASB7439 (each peptide overlaps the next by 11 amino acids). These peptides are set forth in a table found on page 72 of the '872 application. Pages 73-75 then describe the activity of several peptides. Specifically, peptide # 21 is discussed on page 73, lines 12-13; Peptide # 16 is discussed on page 74, line 10; Peptide #'s 23, 24, and 25 are discussed on page 75, lines 3-4. As the reader will note, the sequence for each of these peptides is provided in the table on page 72.

Exhibit B provides a pairwise alignment between the peptides discussed in

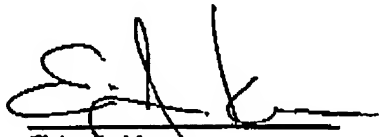
Example 10 and the fragments of SEQ ID NO:2 disclosed in SEQ ID NOS:16-33. The alignment is on the left hand side of the page, with the amino acid residues corresponding to the peptides shaded in gray. In each panel, the peptides are aligned above the sequence of SEQ ID NO:2; fragments disclosed in SEQ ID NOS:16-33 are aligned below SEQ ID NO:2. On the right hand side of the page, Applicants' representative has indicated the Example in which each specific peptide is discussed, as well as the amino acid residues of SEQ ID NO:2 that correspond to each peptide. As the reader will doubtlessly observe, peptides corresponding to amino acid residues 1-14 and 157-172 also appear in the pairwise alignment. These peptides are discussed in Examples 11, 12, and 17 or Examples 12 and 17, respectively.

Applicants hope that these materials will clarify the relationship between the peptides discussed in the examples and the polypeptide fragments set forth in SEQ ID NOS:16-33.

Should the Examiner have further questions or comments with respect to examination of this case, it is respectfully requested that the Examiner telephone the undersigned attorney so that further examination of this application can be expedited.

The Commissioner is hereby authorized to charge any fees due in connection with this paper to Deposit Account No. 07-1392.

Respectfully submitted,



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HLA-DRB5*0101 : nonamers				
Rank	Start Position	Subsequence Residue Listing	Tepitope Score	SEQ ID :
1	96	VEYIRALQR	4.3	SEQ ID NO:32

Example 10

CASB7439 specific cellular immune response

A further way of assessing CASB7439 immunogenicity is to demonstrate CASB7439 antigen has a potential to trigger a cellular immune response. For that purpose, it has to be verified the human CD4+T-cell repertoire has the ability to recognise CASB7439 antigens presented by APC's (antigen-presenting cells) in a MHC class II restricted manner.

To demonstrate CASB7439 antigen can generate a specific CD4+T-cell activity in human, as well as to identify CASB7439 epitopes, a series of "in vitro priming" experiments have been carried out with the PBMC's of three healthy donors.

In vitro Priming of donor #1

In-vitro priming cultures with the PBMC of donor #1 were established using 15-mer peptides overlapping by 11 amino acids from the sequence of CASB7439.

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CD4 peptide table.

Peptide #	Sequence	Amino Acids	Pool
1	MDGGTLPRFAQDMPKD	1-15	1
2	TLGRFAPLVAPFVPVG	5-19	1
3	FAPLVAPFVPVGCDAA	9-23	1
4	VAPFVPVGCAAPSRSA	13-27	1
5	PVGCDAAPSRSAPSPL	17-31	1
6	DAAPSRSAPSPLLRCE	21-35	1
7	SRSAPSPLLRCERAAR	25-39	1
8	PLLRCERAAKVPATA	29-43	2
9	RCERAAKVPATAPTGG	33-47	2
10	AAKVPATAPTGGCAAA	37-51	2
11	ATAPTGGCAAAVASR	41-55	2
12	TGGCAAAVASRNPRS	45-59	2
13	AAAVASRNPRSFPMV	49-63	2
14	ASRNPRSFPMVVK	53-67	2
15	PRSFPMVVKLGTQ	57-71	3
16	PMVVKLGTQGALQ	61-75	3
17	VVKLGTQGALQQVPK	65-79	3
18	GTQGALQQVPKGGAS	69-83	3
19	LQQVPKGGASKKLS	73-87	3
20	VPKGGASKKLSLECVST	77-91	3
21	GASKKLSLECVSTLRSA	81-85	3
22	KLSLECVSTLRSAVSYI	85-99	4
23	VSTLRSAVSYIKAALQ	89-103	4
24	RSAVSYIKAALQKLLA	93-107	4
25	SYIKAALQKLLAEKGA	97-111	4
26	ALQKLLAEKGAVKMA	101-115	4
27	LLAEKGAVKMALAGG	105-119	4
28	KGAVKMALAGGLRPQ	109-123	5
29	KMALAGGLRPQAVKP	113-127	5
30	AGGLRPQAVKPSAPR	117-131	5
31	RPQAVKPSAPRGPPG	121-135	5
32	VKPSAPRGPPGTTPV	125-139	5
33	APRGPPGTTPVAASP	129-143	5
34	PPGTTPVAASPSRAS	133-147	6
35	TPVAASPSRASKSPG	137-151	6
36	ASPSRASKSPGRGGS	141-155	6
37	RASKSPGRGGSSPPG	145-159	6
38	SPGRGGSSPPGSPRS	149-163	6
39	GGSSPPGSPRSAYSS	153-167	6
40	SPGSPRSAYSSDGSG	157-171	7
41	PRSAYSSDGSGGGA	161-175	7
42	YSSDGSGGGALSPA	165-179	7
43	GSGGGALSPAERL	169-183	7
44	GGALSPAERLLDPS	173-187	7
45	SPAERLLDPSSMLGSY	177-193	7

US Serial No. 10/650608,
Atty Docket No. BC45300-1
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Exhibit A
pages 71-75 of
US Serial No. 10/226872

Peptides were combined in pools of 6 or 7 peptides/pool (the resulting 45 peptide sequences and 7 pools are detailed in table 5), and pulsed onto autologous dendritic cells (DCs). Following 4 stimulation cycles, donor #1 PBMC cell lines were assayed for proliferation by using a 3H-thymidine incorporation assay and for IFN-γ synthesis by ELISA.

A number of positive peptide pools were identified, with 21 and 7 cells lines exhibited stimulation index (S.I.)>3 and S.I.>5, respectively (Stimulation index reflects the ratio of activity from T cells incubated with DC pulsed with relevant vs. irrelevant peptide or protein).

All positive 21 lines were further re-stimulated with individual peptides. One line, designated 3H8, showed a specific reactivity to peptide #21.

To map the particular epitope within peptide 21 and recognised by the T-cells of donor #1, T-Cell clones ere individualized from the T-cell lines. For this purpose, the line 3H8 was re-stimulated on antigen expanded using polyclonal activator PHA, and cloned on PHA. Clones were then tested for peptide stimulation in IFN-γ ELSA assays.

Several clones from line 3H8 were shown to recognize peptide 21. Clones generated from this 3H8 line recognised peptide but failed to recognise *E. coli*-derived NS1-CASB7439 protein. Therefore a similar in vitro priming procedure with a new donor has been undertaken to generate T-clones able to recognise the whole CASB7439 protein presented by APC's.

<u>In vitro Priming of donor #2</u>

In vitro priming experiments were preformed with the PBMC from an additional donor #2 in similar experimental condition as donor #1. In brief, PBMC were stimulated with autologous DC pulsed with the 7 pools of 6-7 peptides at a concentration of 250 ng/ml for each peptide. 29 cells lines showed reactivity to pooled peptides, and were further assayed on individual peptides (at 250 ng/ml) and on *E. coli*-derived NS1-CASB7439 protein (10 µg/ml).

5 of these lines (lines 3A3, 4C5, 3C9, 4D5, and 4B12) demonstrated specific reactivity to a particular peptide pool and to both an individual peptide derived from that pool and E. coli-derived NS1-CASB7439.

Two of the lines, 3A3 and 4C5, have been cloned using PHA. The resulting clones were assayed against DCs pulsed with *E. coli*-derived NS1-CASB7439 fusion protein (2.5 µg /ml) or with irrelevant protein (OspA: 2.5 µg /ml) and assayed for proliferation (3H-Thy) as well as IFN-γ production. 61 clones from the two cell lines were shown to recognise *E. coli*-derived protein, and all of the 61 clones have been also shown to recognise the peptide 16 from the pool.

These 16 clones were further characterised by demonstrating MHC Class II restriction. CD4+clones derived from line 3A3 were assayed against DCs pulsed with *E. coli*-derived NS1-CASB7439 protein or irrelevant protein (OspA: 1 µg /ml) in the presence or absence of antibodies (25 µg /ml) to Class I (W632), Class II (HB145), HLA-DR (L243), or HLA-DQw3 (HB144). These T cell clones were shown to be restricted by MHC Class II, and more precisely are not DR DQw3 restricted. Moreover, a preliminary donor mismatch analyses suggest that these clones are likely restricted by HLA-DQ0602.

Specificity of the NS1-CASB7439 fusion protein generated CD4+ T cell activity is further demonstrated: indeed, a line 3A3 clone CD4 response titers out with CASB7439 protein. No response to OspA, an irrelevant protein used as negative control, is observed.

In vitro Priming of donor #3

In vitro priming cultures were established from an additional donor using the same pools of 15-mer peptide overlapping 11 amino acids and the same procedures. Four CD4+lines, including 4A7 and 4E4, demonstrated *E. coli*-derived recombinant protein reactivity that was blocked by antibody to MHC Class II.

Furthermore, CD4+ clones derived from lines 4A7 and 4E4 were assayed against dendritic cells pulsed with *E. coli*-derived NS1-CASB7439 protein or irrelevant protein (OspA: 10 µg /ml), or CASB7439 peptides (250 ng/ml) in the presence or absence of antibodies to Class I (W632:25 µg /ml) or anti-HLA-DR (L243:25 µg /ml). CD4+ clones

derived from these lines present a specificity that is different from the clones descried above, as they are HLA-DR restricted. Moreover, 3 other peptides were shown to be recognised by CD4+T cells (peptides 23, 24, and 25), the total number recognised peptide that were identified being 5 so far.

Example 11

Immunohistochemical analysis of CASB7439 on tumour and normal colon biopsies.

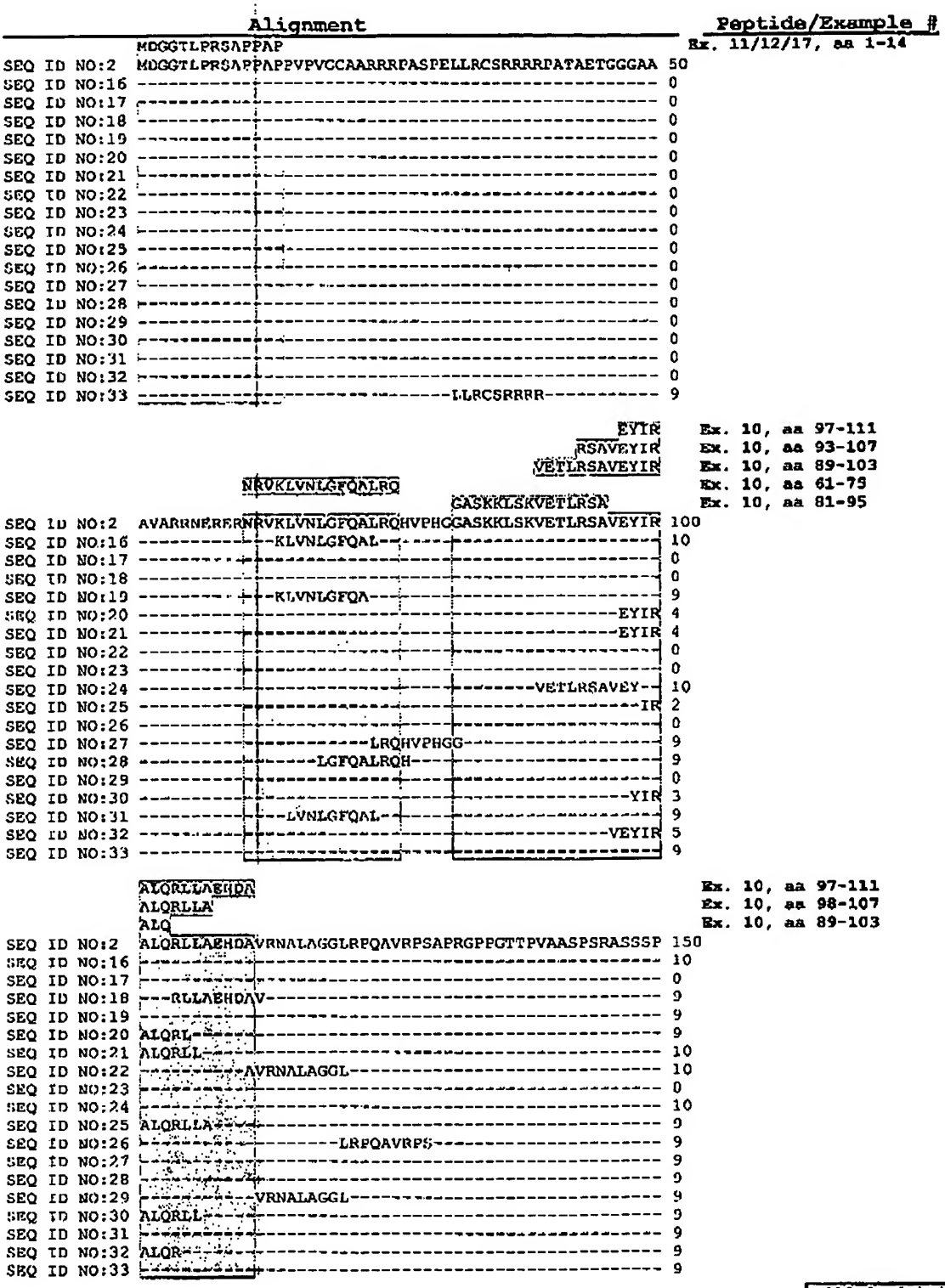
CASB7439 protein over-expression in colon tumour was verified by immunohistochemistry (IHC) using a CASB7439 specific rabbit polyclonal antibody (Ab CASB7439 #599, 1/50 dilution) directed against an affinity purified CASB7439 α-peptide (SB599 α-peptide, amino acids 1 to 14 of CASB7439).

Ab CASB7439 #599 was generated as follows: a rabbit is immunised with SB599 synthetic α-peptide that is conjugated to a carrier protein (LKH). Conjugate is formulated with Freund's adjuvant, and two rabbits are immunised with formulated conjugate. Four weeks after the second immunisation and four weeks after the third immunisation, blood samples are taken. Anti-CASB7439 antibody titers are estimated in the serum by ELISA.

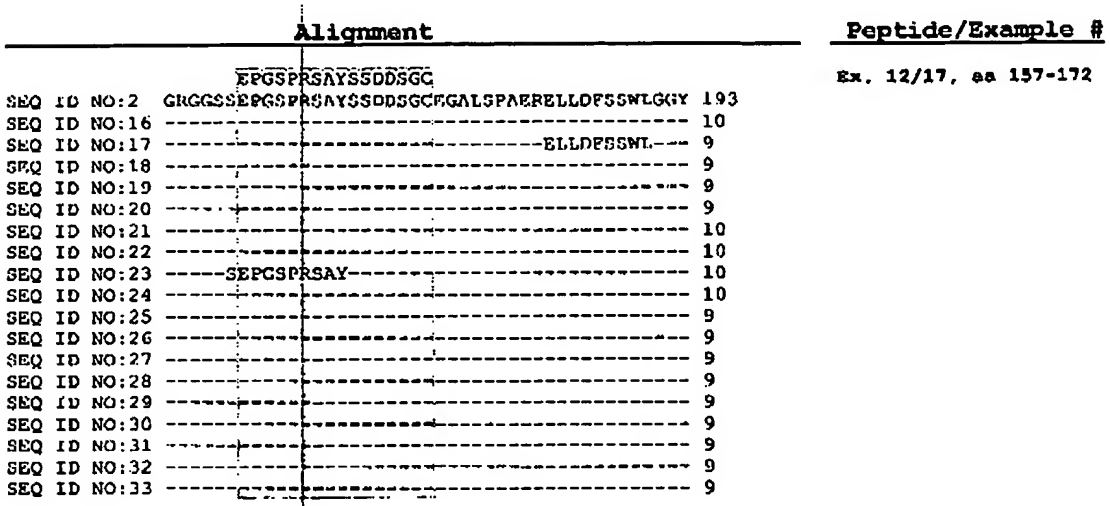
For IHC, paraffin-embedded formalin fixed tissue was sliced into 8 micron sections. Steam heat induced epitope retrieval (SHIER) in 0.1 M sodium citrate buffer (pH 6.0) was used for optimal staining conditions. Sections were incubated with 10% serum/PBS for 5 minutes. Ten micrograms/ml of primary antibody (SB599) was added to each section for 25 min followed by a 25 min incubation with a biotinylated anti-rabbit antibody. Endogenous peroxidase activity was blocked by three 1.5 min incubations with hydrogen peroxidase. The avidin biotin complex/horse radish peroxidase (ABV/HRP) system was used along with DAB chromogen to visualise antigen expression. Slides were counterstained with hematoxylin.

Figure 7 and 8 shows IHC results on colon tumour #9476 biospy and colon normal mucosa #9476, respectively. Anti-CASB7439 immunoreactivity was observed at high level in colon cancer and in normal colon at very low level. Anti-CASB7439

Pairwise Alignment Between SEQ ID NO:2, SEQ ID NOS:16-33, and Peptides of Example Numbers 10, 11, 12, and 17



Pairwise Alignment Between SEQ ID NO:2, SEQ ID NOS:16-33, and Peptides of Example Numbers 10, 11, 12, and 17



US Serial No. 10/650608,
Atty Docket No. BC45300-1
26 Sep 2006Communication
Exhibit B
Pairwise Alignment